Dengue Serotyping Diagnostic Kit (PCR-Fluorescence)

REF 106-0043-01

Product Name
Dengue Serotyping Diagnostic Kit (PCR-Fluorescence)

Specification
12 reactions/kit

Intended Use
Dengue fever is an acute infectious disease caused by dengue virus (DENV) and is one of the most widely spread mosquito-borne infections worldwide.
There are four serotypes of dengue virus, namely DENV-1, DENV-2, DENV-3, and DENV-4, which mainly rely on the vector Aedes mosquitoes for transmission, which can continue to spread between the human population and the vector Aedes mosquitoes Circulation, leading to outbreaks of epidemics.
After being infected with one dengue virus, the human body will develop a lasting immunity to the same type of virus, but cannot form effective protection against different types of virus infection. Re-infection with different types of DENV will trigger an increase in non-neutralizing cross-reactive antibodies and cause antibody-dependent enhancement (ADE), which is an important cause of severe dengue fever. The clinical manifestations of severe dengue fever are severe hemorrhage, shock, and severe organ damage. Therefore, there is a certain clinical reference significance for the typing of dengue virus.
This kit utilizes RT- PCR to qualitatively analyze serum samples for the four serotypes of dengue virus (DENV), and the results are for clinical reference only.

Test Principle
The kit uses iCassette technology in combination with supporting instruments to automatically perform nucleic acid extraction and nucleic acid amplification by the instrument throughout the entire process, reducing direct cross-contamination of samples. At the same time, a built-in QR code scanner can automatically identify the execution process corresponding to this kit. A uniquely designed software of the kit is used to perform nucleic acid extraction, whole-process PCR, result display and analysis. The Kit contains twelve sets of DENV iCassette, which includes nucleic acid extraction reagents and PCR reagents. For a complete description of the instrument, please refer to the instruction for use of the corresponding instrument.
The Kit adopts the real-time multiplex fluorescent real-time PCR technology to qualitatively detect the DENV-1, DENV-2, DENV-3 and DENV-4 in patients' serum samples in vitro. This product designs primers and fluorescent probes for detection based on DENV-1, DENV-2, DENV-3 and DENV-4, and also designs primers and probes for internal control. Through the detection of the quality control sample, confirm whether the target virus is fully processed, and detect whether there are PCR reaction inhibitors.

Components

Kit Components			Content	Quantity
DENV iCassette (12 pcs)	Nucleic acid extraction reagent	Virus lyophilization A	Proteinase K	1 pc/iCassette
		DENV lyophilization B	Pseudovirus lyophilization containing internal control fragments	1 pc/iCassette
		Virus lysate solution	Guanidinium isothiocyanate	450 μL/iCassette
		Virus binding solution	Guanidinium isothiocyanate	175 μL/iCassette
		Virus washing solution	Sodium Chloride	900 μL/iCassette
		Virus eluent	Tris-HCl	90 μL/iCassette
		Magnetic beads	Magnetic Microspheres	11 μL/iCassette
		DENV PCR cosolvent solution	PCR Buffer, $MgCl_2$	40 μL/iCassette
	DENV PCR reagent	DENV lyophilization	Specific primer, probe, dNTP, enzyme	1 pc/iCassette
Control	DENV positive control		Pseudovirus with target fragments	1tube(200μL)
	DENV negative control		Sterilized PW	1tube(200μL)

Note:Components in kits with different batch numbers are not interchangeable

Storage condition and Shelf life
1. The kit can be stored at 2-8℃ and the shelf life is 9 months.
2. The transportation temperature range of the kit should be kept at 2-8℃.
3. Please do not open the iCassette lid before adding the sample. If you open the iCassette lid, it should be used within 30 minutes.

Applicable instruments
Automated Fully Enclosed qPCR Instrument: Galaxy Lite and Galaxy Pro.

Materials Required but Not Provided
- Automated Fully Enclosed qPCR Instrument : Galaxy Nano, Galaxy Lite or Galaxy Pro.
- Leak-proof, sterile, screw-capped specimen collection containers.
- Disposable gloves, eye protection, laboratory coats, and labels or permanent marking pen.
- Centrifuge.
- Pipettes.
- Sterile pipette tips.

Sample Requirements
1.Applicable sample types: human serum sample.
2. Sample collection
Serum sample: Use sterile vacuum drying tubes to collect non-anticoagulated blood from patients, separate serum in time, and store in separate packages in cryopreservation tubes with screw caps and gaskets inside, and store at low temperature after labeling.
3.Sample storage and transportation
Samples used for virus isolation and nucleic acid testing should be tested as soon as possible. Samples that can be tested within 24 hours can be stored at 2-8°C; samples that cannot be tested within 24 hours should be stored at -70°C or below (or in a refrigerator at -20±5°C if there is no storage at -70°C. Blood specimens can be stored at -20℃ or below, but not more than 1 week).
4. Principles of Bio-safety Protection
All operations shall comply with relevant local laws and regulations.

Test Method and Operation
1. Prepare DENV iCassette
1.1. 1.Processing samples or control materials in the samples preparation room, Serum samples were collected from patients with 5 mL of non-anticoagulated blood. Centrifuge at 3000rpm for 5 minutes, then carefully transfer the supernatant to cryovials with a sterile pipette.
1.2 Open the package of DENV lyophilization, observe whether the DENV lyophilization is intact, replace the empty PCR tube on the iCassette with the PCR tube containing the DENV lyophilization, and make sure the PCR tube is screwed up.
1.3 Open the lid of the iCassette, and pipette 200 μl of sample or controls to the DENV iCassette sample compartment as shown in Figure 1. slowly, close the lid tightly.(Note: After the sample is added to the iCassette, the test needs to start running within 30 minutes)
1.4 Place the iCassette into the instrument.

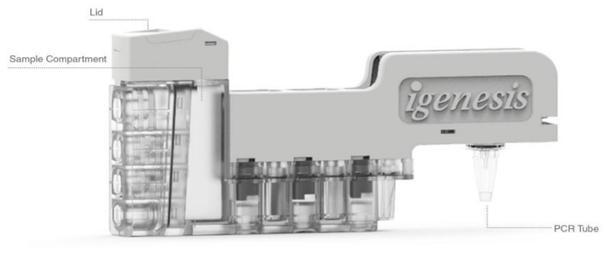

Figure 1 DENV iCassette (Lateral View)

2. Test Operation
2.1 Turn on the power, press the switch button on front side of the instrument, and the blue light is on, i.e. "the instrument is on". After the instrument is turned on, click the control software icon on the desktop to enter the login interface.
2.2 Log in to the software for the first time with the administrator account (Admin/123456), click "OK" to complete the login.
2.3 Click the "Open" button in the initial interface to open the compartment door of the instrument.
2.4 Put the iCassette that has been added with sample into the instrument and click the "Close" button in the initial interface to close the compartment door. (For multi-channel instrument, iCassette tray holder is required to load the iCassette. Input the sample information at the corresponding channel position of the control software while loading the iCassette)
2.5 After the compartment door is closed, the instrument scans the corresponding QR code on the iCassette automatically.
2.6 After scanning, click the "Run" button, the software will automatically load the program corresponding to the QR code of the iCassette, and click "OK" to start the program.
2.7 After the program starts , the progress of the instrument running will be displayed in the main interface.
2.8 After the amplification is completed, the compartment door will open automatically.
For the detailed steps of test operation, please refer to user manual of the instrument.

3. Result Analysis
After the experiment is finished, the instrument will automatically save the results, and output the Ct value and amplification curves with results interpretation in the interface.

Quality Control
Test results of positive control: FAM channel, HEX channel, TEXAS RED channel,CY5.5 channel and CY5 channel are all positive;
Test results of negative control: FAM channel, HEX channel, TEXAS RED channel, CY5.5 channel are all negative; CY5 channel is positive;
Internal control: CY5 channel is positive (internal control may be detected as negative when the sample to be tested is positive due to the specific competition between the internal control and the sample. When the clinical sample is negative, the internal control must be positive, otherwise it may be due to sampling failure or extraction failed).
The above requirements must be met at the same time in the same test. Otherwise, the test is invalid and needs to be repeated.

Interpretation of Test Results

Negative	No Ct value or Ct > 29
Positive	Ct ≤ 27
Suspicious	If the Ct value falls 27-29, it is recommended to repeat the test. If the Ct < 27 again and the amplification curve has obvious peaks, the sample is judged as positive, otherwise it is negative.

The sample to be tested will be determined according to the standards in the table below:

FAM Channel	HEX Channel	TEXAS RED Channel	CY5.5 Channel	CY5 Channel	Results
√	×	×	×	√	DENV-1 positive(Figure 2)
×	√	×	×	√	DENV-2 positive(Figure 3)
×	×	√	×	√	DENV-3 positive(Figure 4)
×	×	×	√	√	DENV-4 positive(Figure 5)
√	√	×	×	√	DENV-1 and DENV-2 positive
√	×	√	×	√	DENV-1 and DENV-3 positive
√	×	×	√	√	DENV-1 and DENV-4positive
×	√	√	×	√	DENV-2 and DENV-3 positive
×	√	×	√	√	DENV-2 and DENV-4 positive
×	×	√	√	√	DENV-3 and DENV-4 positive
√	√	√	×	√	DENV-1, DENV-2 and DENV-3 positive
√	√	×	√	√	DENV-1, DENV-2 and DENV-4 positive
√	×	√	√	√	DENV-1, DENV-3 and DENV-4 positive
×	√	√	√	√	DENV-2, DENV-3 and DENV-4 positive
√	√	√	√	√	DENV-1, DENV-2, DENV-3 and DENV-4 positive
×	×	×	×	√	Negative control
√	√	√	√	√	Positive quality

Note:"√" means that the result "has an obvious logarithmic amplification curve"; "×" indicates the result has "no logarithmic amplification curve". The CY5 channel is an internal control channel.

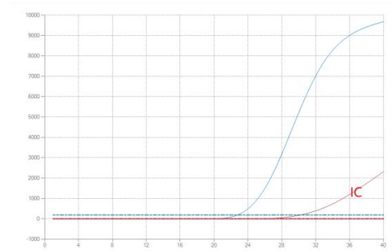

Figure 2. DENV-1 Positive

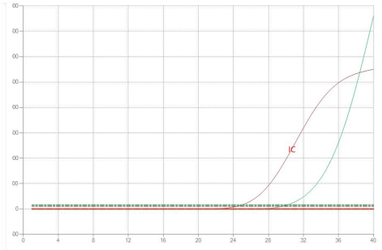

Figure 3. DENV-2 Positive

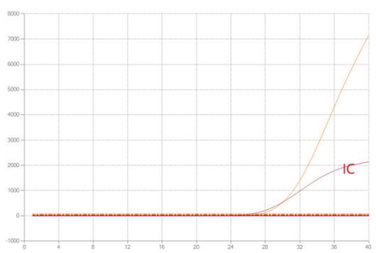

Figure 4. DENV-3 Positive

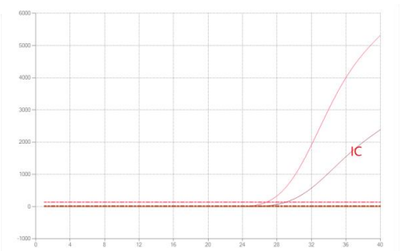

Figure 5. DENV-4 Postive

Limitations of Test Method

1. Improper sample collection, transportation and processing, too low virus content in the sample, excessive nucleic acid degradation or target concentrations below the LOD in the amplification reaction system may lead to false negative results.
2. The test results of this product cannot be used directly as the basis for clinical diagnosis or case exclusion. However, they should be analyzed comprehensively in combination with other relevant medical test results.

Product Performance Index

1. The lower limit of detection of the Kit: 1×10^3 copies/mL.
2. Analysis specificity

The specificity test results show that there is no cross reaction with pathogenic microorganisms (Japanese encephalitis virus, tick-borne encephalitis virus, yellow fever virus, Hepatitis C virus; influenza B virus lineages Yamagata and Victoria; influenza A virus subtypes H1N1, H3N2, H5N1, H7N9; EBV, measles virus, rubella virus) same as those at the infection site or causing the similar symptoms of infection.

3. Potential interfering substances

Endogenous substances in human serum such as whole blood and mucus don't interfere with the test results of the Kit. Substances (anticoagulants, coagulants, separating gels) in the blood collection tubes and exogenous drugs (ribavirin, amoxicillin, dexamethasone, paracetamol, ibuprofen, amantadine, etc.) don't interfere with the test results of the Kit.

4. Precision:

The coefficient of variation of intra-batch precision is ≤ 5%.

Precautions

1. The kit should be used within the validity period.
2. If the iCassette is leaked after adding the sample, do not use the iCassette.
3. Experimenters should take protection and wear disposable gloves and masks.
4. Each iCassette is a single-used, please do not reuse it.
5. In order to avoid any potential biological hazards in the samples, the test samples should be regarded as infectious substances and avoid contact with skin and mucous membranes; sample handling and processing must comply with relevant regulatory requirements.

References

1. Leta S, Beyene TJ, De Clercq E, et al. Global risk mapping for major diseases transmitted by Aedes aegypti and Aedes albopictus [J]. International Journal of Infectious Diseases, 2017, 67(C) DOI: 10.1016/j.ijid.2017.11.026.
2. XY Cheng. Contribution of phylogenetics to understanding the evolution and epidemiology of the dengue virus [J]. Journal of Power and Mechanical Model Experimental Medicine (English), 2022, 5(5): 410-417.

Instruction Version

Version: A/0
Date of Issue:March,2023

Symbols

The following symbols may appear on the product labeling:

IVD	In vitro diagnostic medical device		Do not re-use
	Use-by date		Consult instructions for use or consult electronic instructions for use
	Caution		Manufacturer
	Temperature limit	LOT	Batch code
	Contains sufficient for <n>		Keep dry
	Keep away from sunlight		Do not use if package is damaged and consult instructions for use
	Date of manufacture		Biological risks
REF	Catalogue number	CE	CE marking of conformity
EC REP	Authorized representative in the European Community		

IGENESIS(SHANGHAI)CO. , LTD.
Address: Floor 3, building 1, Lane 500, Furonghua Road, Pudong New Area, 201318 Shanghai, P.R. China.
Tel: +86-21-38016598

EC REP **Riomavix S.L.**
Address: Calle de Almansa 55, 1D, Madrid 28039 Spain
E-mail: leis@riomavix.com
Tel.: +34 658 396 230